Supplemental Material

Supplemental table 1: Circulating P-3-OHB levels, blood sample measurement and administered volume in each study arm of the PET studies

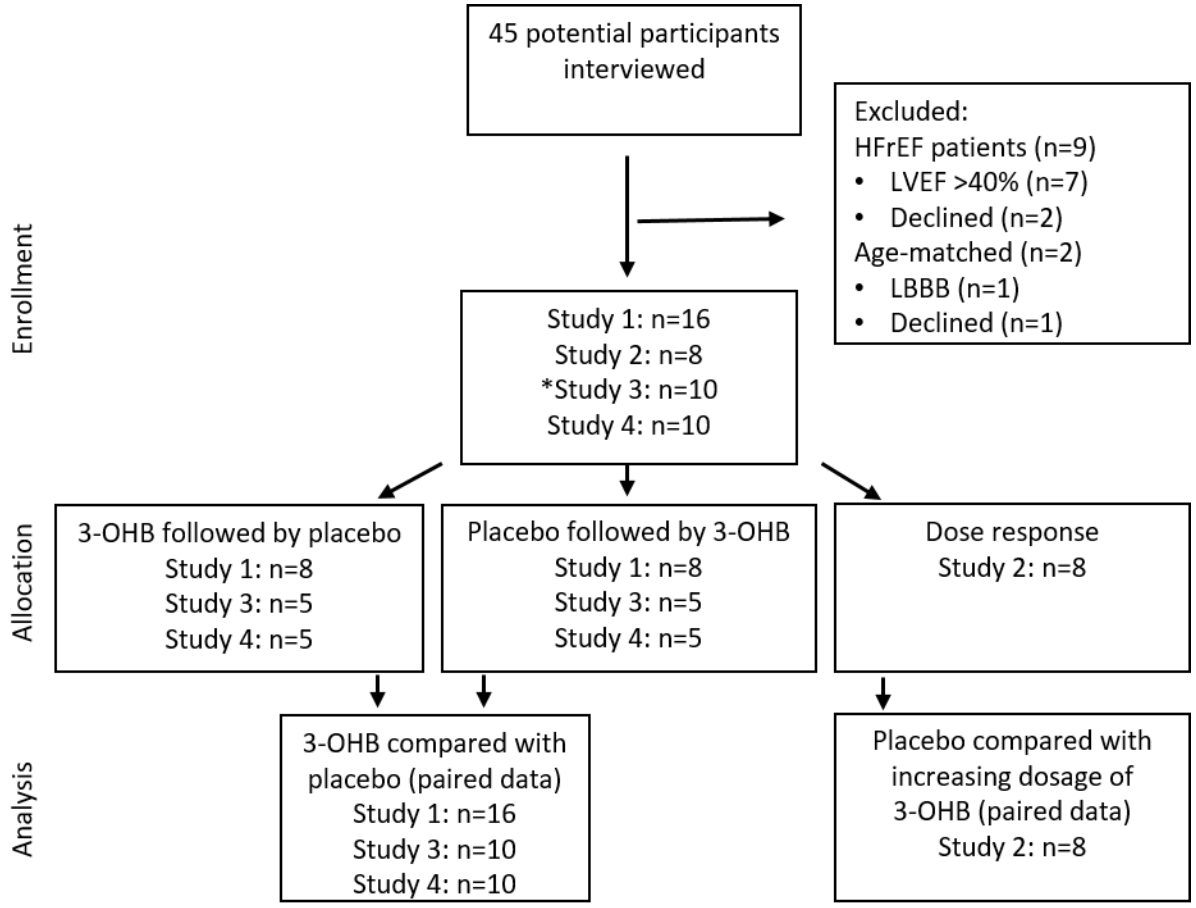
	HFrEF patients (n=10) (Study 3)		Age-matched volunteers (n=10) (Study 4)		P-value (3-way repeat. ANOVA)	Inter-action (group)	Inter-action (inf. seq.)
	Saline	3-OHB	Saline	3-OHB			
3-OHB (mM)	0.42±0.37	3.52±0.50	0.30±0.23	3.2+±0.60	**<0.001**	0.43	0.05
pH	7.39±0.05	7.43±0.03	7.40±0.02	7.45±0.02	**<0.001**	0.25	**<0.001**
Potassium (mM)	4.26±0.21	3.93±0.25	3.91±0.38	3.63±0.35	**0.003**	0.64	**0.001**
Glucose (mM)	5.86±0.38	5.60±0.35	5.64±0.43	5.79±0.55	0.67	0.31	0.65
Lactate (mM)	0.83±0.19	1.33±0.37	1.10±0.25	1.33±0.42	**0.002**	0.45	0.12
FFA (mM)	0.09 [0.02;0.16]	0.04 [0.03;0.10]	0.08 [0.04;0.17]	0.04 [0.03;0.05]	**0.009**	0.72	**0.01**
GIR (mg/kg/min)	3.28±1.91	3.49±2.29	4.76±3.27	5.02±3.72	0.11	0.72	**0.002**
Insulin (pmol/L)	178±44	200±71	152±53	139±55	0.18	0.26	**0.03**
BNP (pmol/L)	35[13;56]	36 [10;64]	7.7[3.7;14.2]	7.5 [3.5;13.8]	0.05	0.50	**<0.001**
Noradrenaline (pg/L)	391±91	417±151	363±103	404±95	0.14	0.50	**0.01**
Adrenaline (pg/L)	32 ±19	34±16	38±13	28±8	0.04	**0.01**	0.07
INFUSED VOLUMES							
Glucose volume (ml)	161 [105; 255]	265 [118;365]	225 [170; 390]	240 [205; 595]	0.04	0.80	**<0.001**
Ketone/saline volume (ml)	630±78	675±158	714±172	677±192	0.63	0.11	0.25

Supplemental table 1: Blood sample measurements during 11C-Acetate-PET for both study groups. Data are mean ± standard deviation or median [interquartile range]

Blood was sampled at the end of each intervention period. Three-way repeated ANOVA analysis was performed and interaction (study group vs treatment and infusion sequence vs. treatment) was examined.

3-OHB: 3-betahydroxybutyrate. FFA: Free fatty acids. GIR: glucose infusion rate. BNP: Brain Natriuretic Peptide.

Supplemental figure 1: CONSORT diagram



Supplement figure 1: All enrolled participants completed the studies.

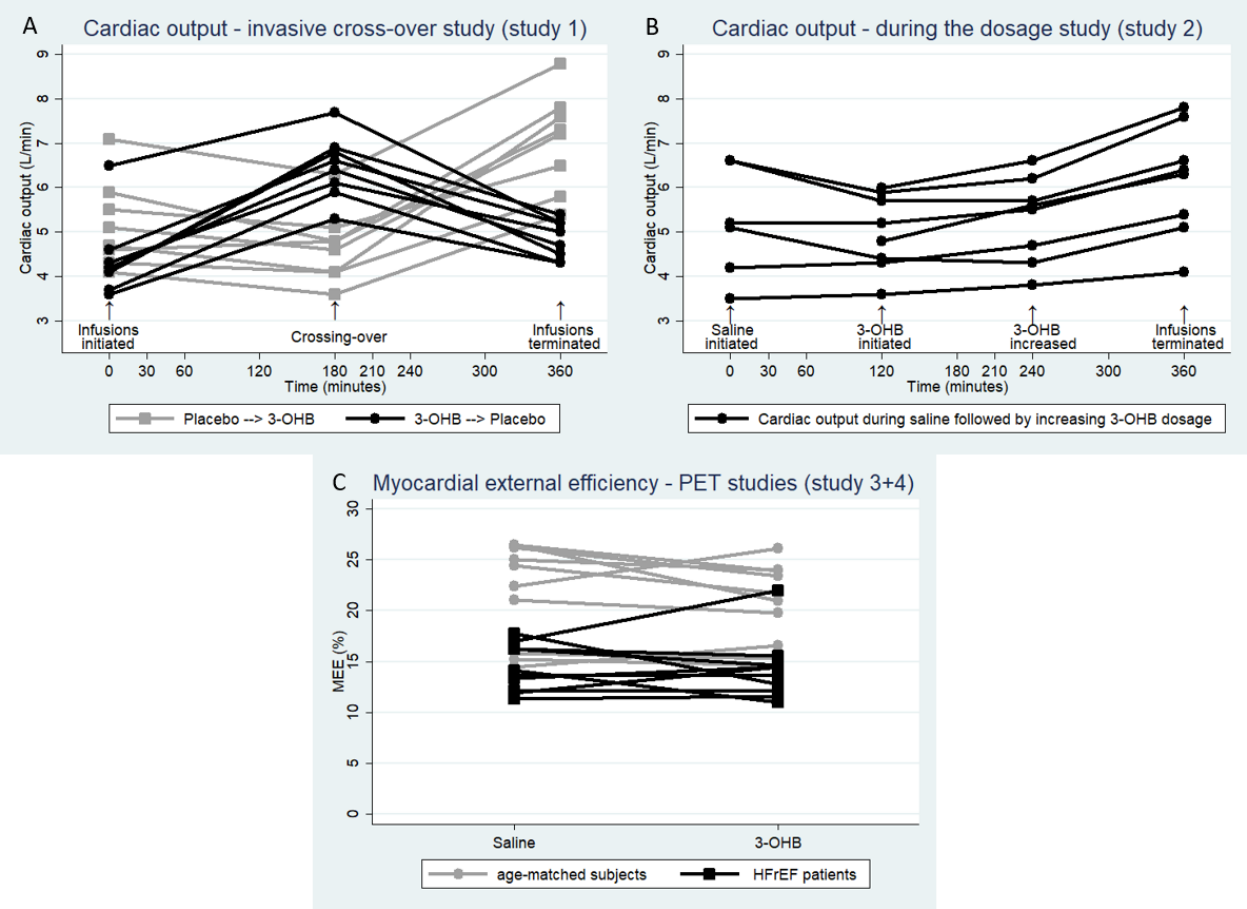
HFrEF: Heart failure with reduced ejection fraction. LVEF: Left ventricular ejection fraction.

LBBB: Left bundle branch block. 3-OHB: 3-betahydroxybutyrate.

*Participants enrolled in Study 3 were recruited consecutively from Study 1.

3-OHB: 3-betahydroxybutyrate

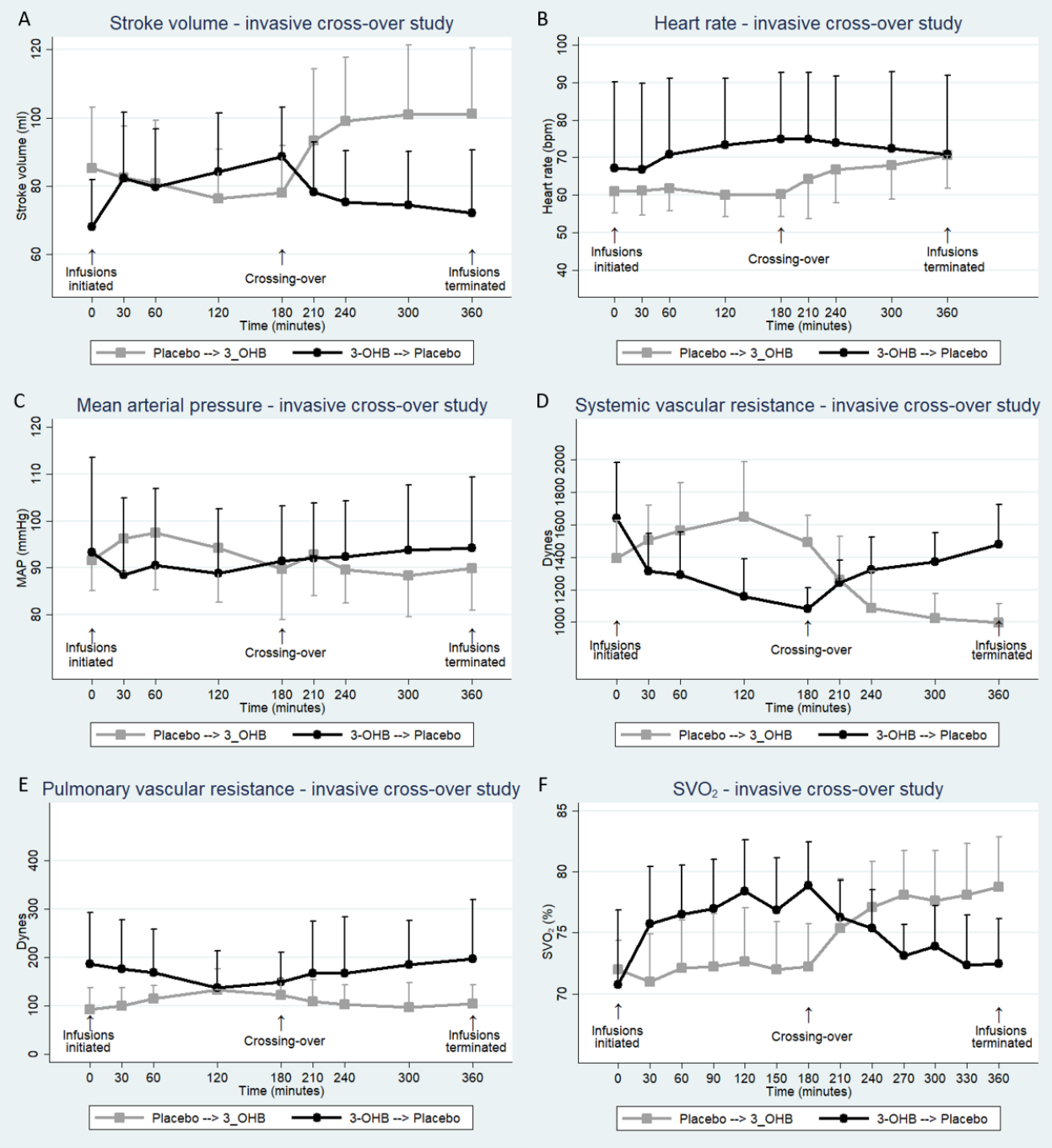
Supplemental figure 2: Changes in primary outcome for each of the four substudies – individual data



Supplemental figure 2: (A) Cardiac output during study 1 - the timing of infusion initiation, cross-over and termination are depicted. (B) Cardiac output during study 2 - the timing of saline infusion, initiation of 3-OHB infusion (0.045 g(kg/h), increase of infusion rate (0.09 g/kg/h) and termination of the infusion are depicted (after completion of the first two participants is was decided to perform full haemodynamic assessment in all participants prior to the intervention). (C) Changes in myocardial external efficiency (MEE) in study 3 (HFrEF patients) and study 4 (age-matched subjects). In study 1 and 2 the endpoints were measured repeatedly during the

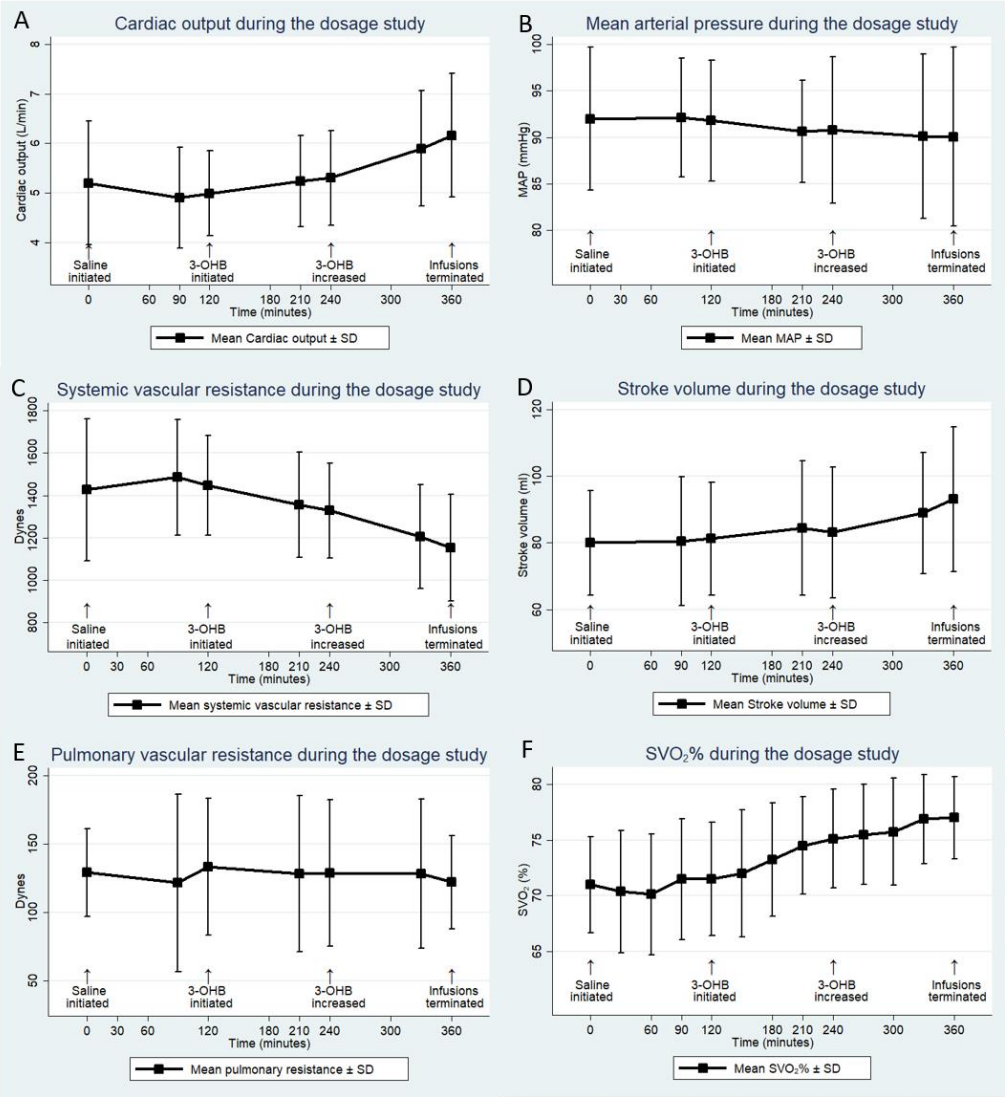
interventions, whereas, in the PET studies (study 3+4), the endpoints were measured at the end of each intervention period. Study 1, 3 and 4 were randomised cross-over studies.

Supplemental figure 3: Haemodynamic changes during the invasive cross-over study (study 1)



Supplemental figure 3: (A) Stroke volume, (B) heart rate, (C) mean arterial pressure (MAP) and (D) Systemic vascular resistance, (E) pulmonary vascular resistance, (F) SVO_2 levels during the invasive hemodynamic cross-over study (mean with bars indicating standard deviation). The timing of infusion initiation, cross-over and termination are depicted.

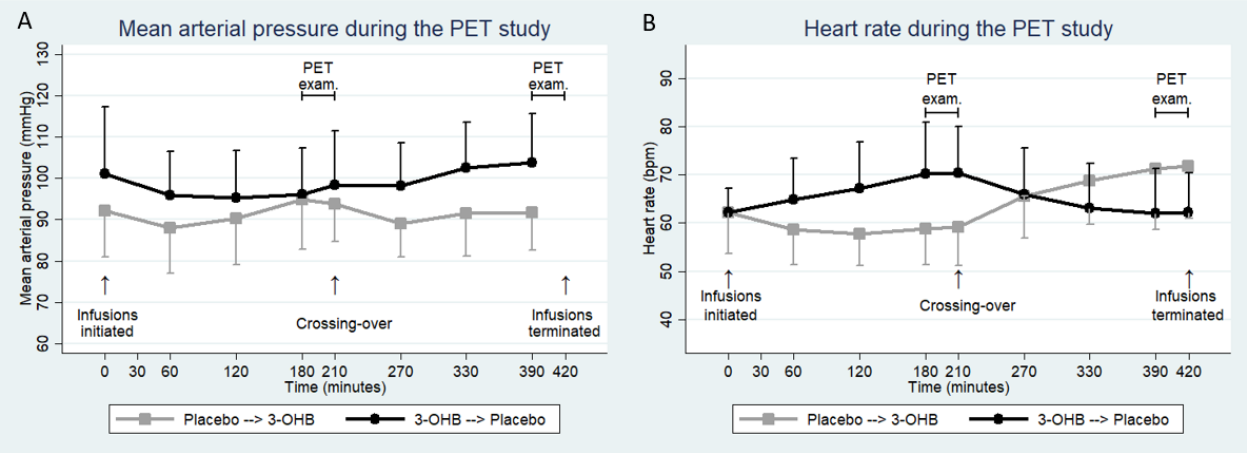
Supplemental figure 4: Invasive haemodynamic data during the dose-response study (study 2)



Supplemental figure 4: (A) Cardiac output, (B) mean arterial pressure, (C) systemic vascular resistance, (D) stroke volume, (E) pulmonary vascular resistance and (F) SVO_2 for the participants in the dose-response study (study 2) (mean with bars indicating standard deviation). The timing of saline infusion, initiation of 3-OHB infusion (0.045 g(kg/h), increase of infusion rate (0.09 g/kg/h) and termination of the infusion are depicted.

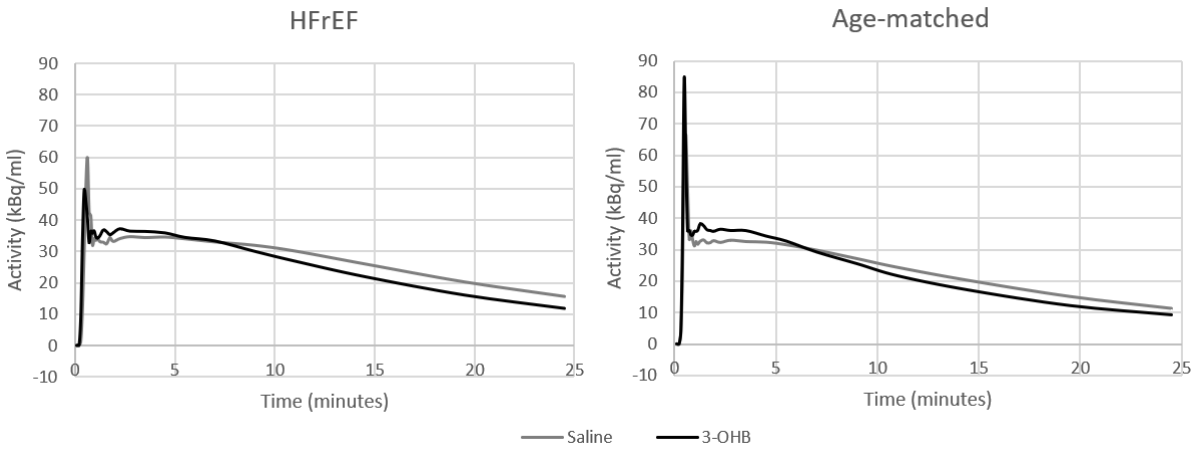
(MAP: mean arterial pressure)

Supplemental figure 5: Changes in blood pressure and heart rate during the PET study (study 3+4)



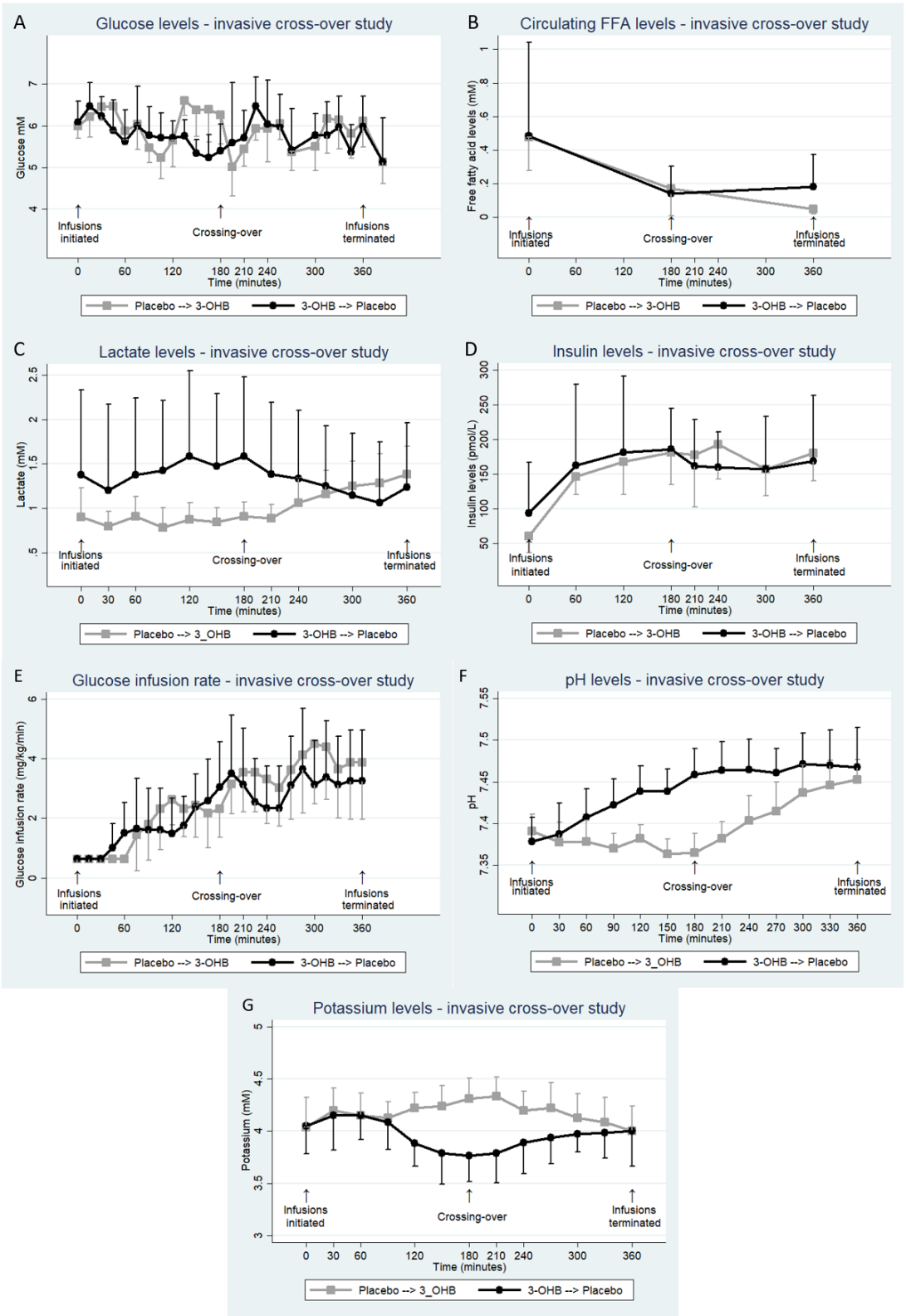
Supplemental figure 5: (A) Mean arterial blood pressure and (B) heart rate (bpm) during the positron tomography study (study 3+4) (mean with bars indicating standard deviation). Timing of infusions, crossing over, termination and PET examinations are depicted.

Supplemental Figure 6: Time-activity curves from a HFrEF patient and an age-matched volunteer



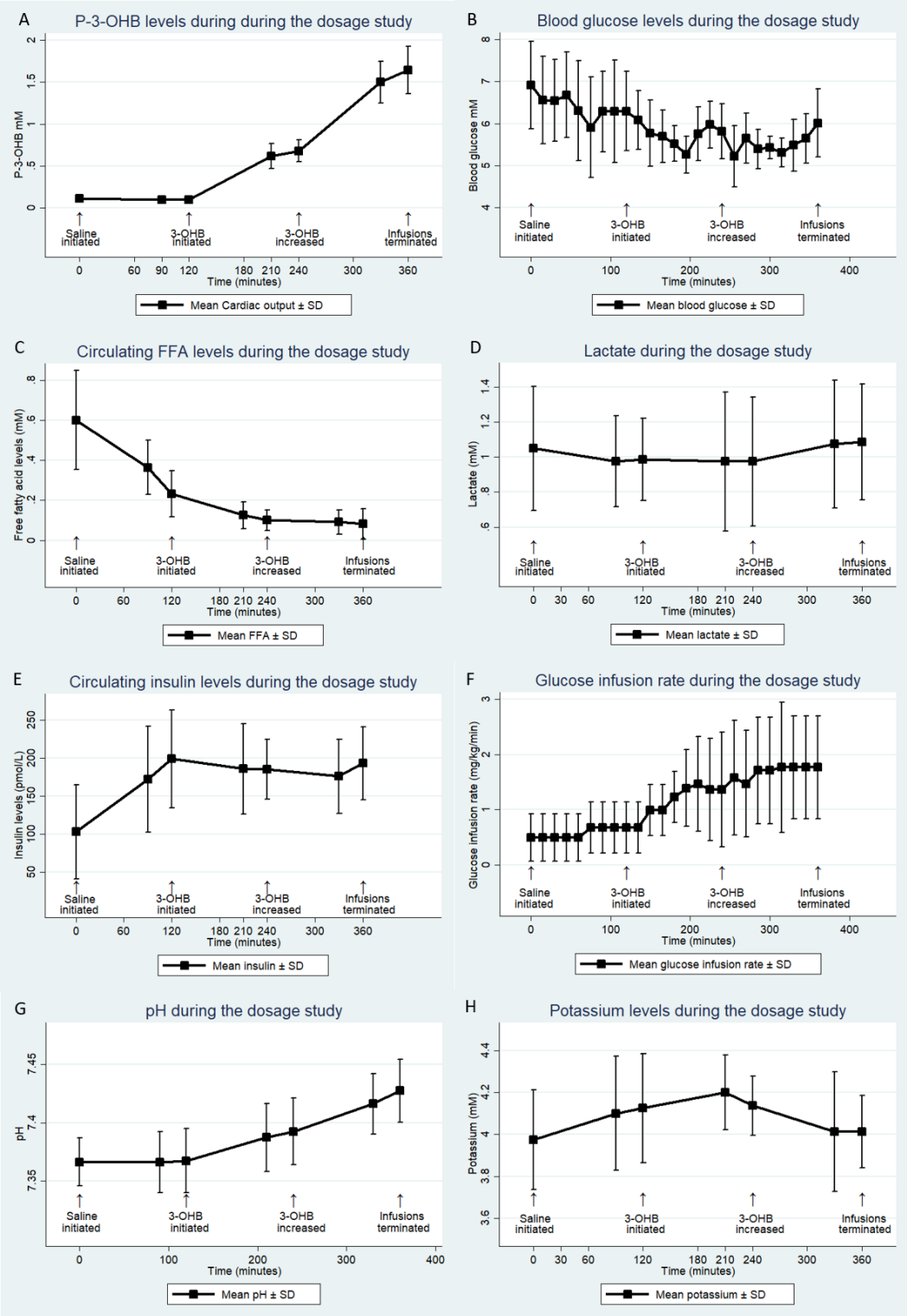
Supplemental figure 6: Examples of time-activity curves (TAC) from 2 participants subjected to 11C-acetate-PET. The curves demonstrate the activity in the myocardium (y-axis) as a function of time (x-axis) after injection of the tracer (11C-acetate) during placebo (Saline) and 3-OHB infusion. The left figure is TAC from a HFrEF patient (LVEF: 38%) and the right figure from a age-matched volunteer (LVEF: 60%).

Supplemental figure 7: Metabolic changes during the invasive cross-over study (study 1)



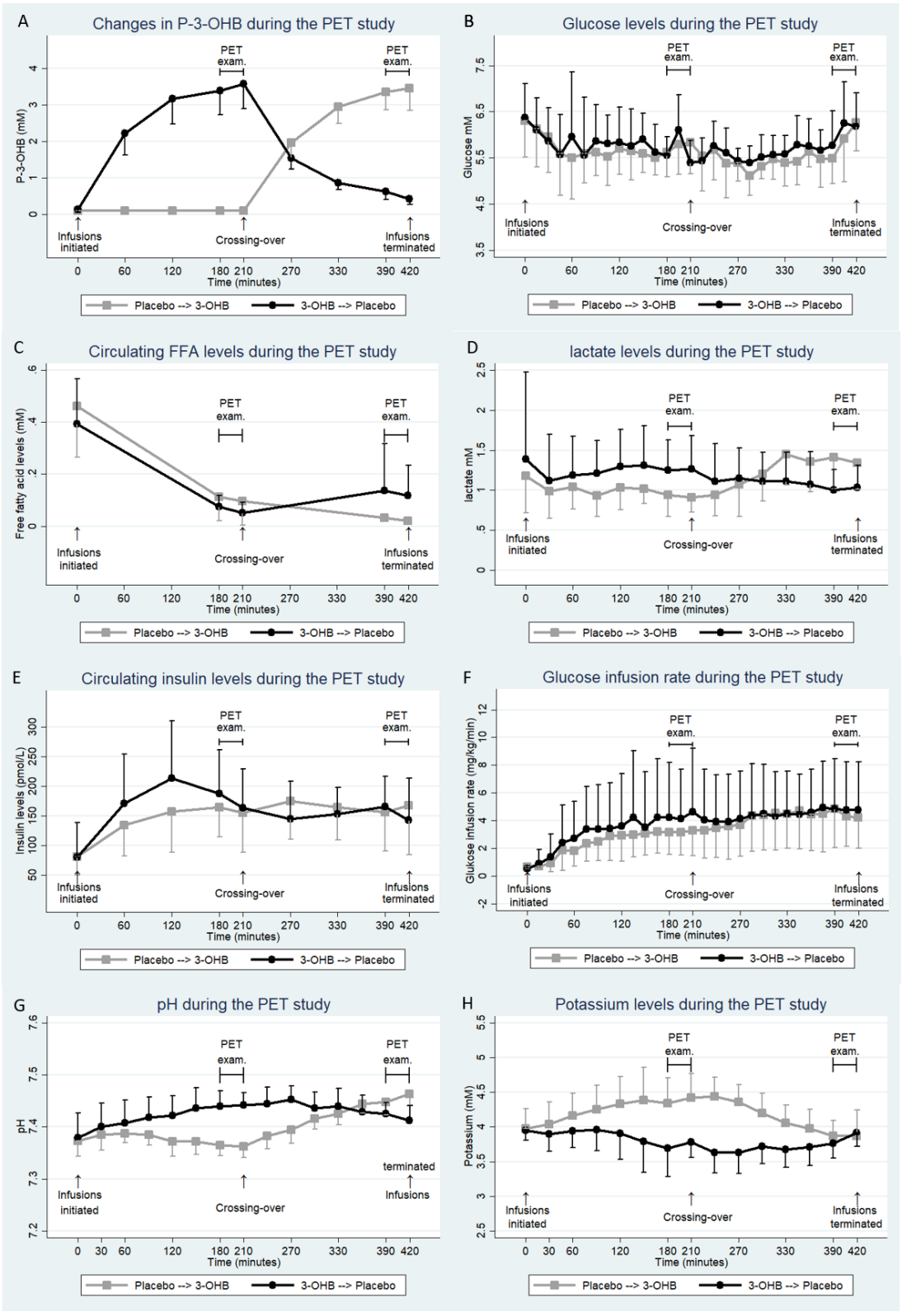
Supplemental figure 7: (A) Glucose, (B) free fatty acids (FFA), (C) lactate and (D) insulin, (E) glucose disposal rate, (F) pH and (G) potassium levels for the invasive hemodynamic cross-over study (mean with bars indicating standard deviation). The timing of infusion initiation, cross-over and termination are depicted. (Isotonic glucose was administered to measure cardiac output and not taken into consideration during calculation of glucose administration. Consequently, the glucose infusion rate should be interpreted with caution).

Supplemental figure 8: Changes in blood sample measurements during the dose response study (study 2)



Supplemental figure 8: (A) P-3-OHB, (B) blood glucose, (C) circulating free fatty acids (FFA), (D) lactate, (E) insulin, (F) glucose disposal rate, (G) pH and (H) potassium levels during the dose-response study (study 2) (mean with bars indicating standard deviation or median with bars indicating IQR (glucose disposal rate)). The timing of saline infusion, initiation of 3-OHB infusion (0.045 g(kg/h), increase of infusion rate (0.09 g/kg/h) and termination of the infusion are depicted. (During the study, isotonic glucose was administered to measure cardiac output and not taken into consideration during calculation of glucose administration. Consequently, the glucose infusion rate should be interpreted with caution).

Suppl. Fig. 9: Changes in blood sample measurements during the PET studies (study3+4)



Supplemental figure 9: (A) P-3-OHB, (B) blood glucose, (C) circulating free fatty acids (FFA), (D) lactate, (E) insulin, (F) glucose disposal rate, (G) pH and (H) potassium levels during the positron tomography study (study 3+4) (mean with bars indicating standard deviation). Timing of infusions, crossing over, termination and PET examinations are depicted.